TOBACCO INDUSTRY RESEARCH COMMITTEE

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"The Effect of Nicotine on the Blood Cholesterol and Phospholipids, the Ergonovine-Modified ECG and Vascular Pathology of Cholesterol-Fed Rabbits."

Experimental Procedure:

Six groups of twelve albine, New Zealand, six-week old, female rabbits per group were established. All animals weighed between 1.7 and 2.1 kilograms. Group I received the stock diet of Purina Rabbit Chow[R] and water ad libitum. For Group II the food was impregnated with cholesterol 1% and cottonseed oil 5%. Group III was fed the stock diet plus the human nicotine equivalent by body weight of two packs of cigarettes daily in the drinking water. Details of this determination have been reported. Groups IV, V, and VI received the Group II diet and 1/8, 1/2, and 2 "pack equivalents" of nicotine respectively. In order to reduce the possibility of acute effects from the nicotine, the dose was gradually increased in order to produce tolerance.

Determinations of body weight, serum cholesterol and phospholipid and electrocardiographic activity were made initially and every eight weeks thereafter for a period of 24 weeks.

Electrocardiography was performed using an Edin C.C.-D.C. amplifier and a Brush Model BL-201 oscillograph at a paper speed of 25 mm./second. The usual limb leads and a chest lead positioned over the heart were used. Electrodes were prepared from silvered hypodermic needles. The rabbits were unanesthetized but were immobilized by use of a rabbit board through which the head could be lowered below the plane of the body. Records were obtained prior to and at one, three, five and ten minutes following the injection of ergonovine maleate into the marginal ear vein.

At 24 weeks all surviving animals were sacrificed and the degree of aortic sclerosis grossly graded on a modified 0 to 4 scale. Grade 0 was considered to be less than 2 percent of the total surface involved; grade 1, up to 10 percent; grade 2, up to 20 percent; grade 3, up to 40 percent; and grade 4, up to 80 percent. When significant

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thickening of the lesions were observed 0.5 was added to the grade. All values were equated to a maximal value of 4 by direct proportion. For the purpose of grading, the aorta was divided into arch, ascending, and thoracic aortic areas.

The heart and aorta was fixed in ten percent formalin. Tissues were stained with oil-red-O and hematoxylin counterstain and frozen sections made through the arch of the aorta and the base and apex of the heart.

Results:

Body Weight. - The mean body weights of all groups are given in figure 1. Weights within groups were fairly uniform, the standard errors ranging from one to ten percent of the group mean weights. All Cholesterol-fed groups lost weight after the 16-week period.

Serum Cholesterol-Lipid Phosphorus. - Figures 2 and 3 illustrate the changes in the serum cholesterol and lipid phosphorus levels. Although an initial linear dose relationship occurs for the nicotine and the serum cholesterol levels, this did not continue, nor was it significant due to large individual variations. Values for the "1/2 pack" group are not given after the 16th week because of the high mortality in this group between 16 and 24 weeks.

Mortality. - Mortality is shown in figure 4. Most of the animals dying were lost between the 16th and 24th-week test periods. Although there were indications of circulatory failure in those animals who died, it was not attempted to correlate the deaths with cardiac involvement. The nicotine-cholesterol groups demonstrated significantly greater mortalities than any of the other groups ($p < 0.01$ except for Group VI, $p < p.05$).

Ergonovine Stress Test. - The electrocardiograms of the cholesterol and nicotine-cholesterol groups did not begin to demonstrate increased abnormalities related to the administration of ergonovine until the 16th week. The results as given in Table I are obtained from the 24-week records. Two criteria of ergonovine stress were selected: A was an ergonovine-induced S-T depression of 1. Omm. (2 cm.= 1 mV) below the isoelectric level and (or) a flattening or inversion of the T-wave in leads II, III, or IV. Criterion B was the same as that of A except that flattening of the T-wave was eliminated as a positive sign of coronary insufficiency. It was felt that Criterion A was more valid as a number of animals in all groups demonstrated the

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flattened T-wave prior to ergonovine stress. The effect of both pentobarbital anesthesia and the position of the animal on the positivity of the electrocardiogram was determined in a number of cases. It was observed that pentobarbitalization had no effect on positivity whereas the position, that is on the back as used in the present study or the side as used by Rinzler[1], markedly affected the results. Animals which were negative in the back position were often positive on the side and vice versa. It may be seen from Table I that a consistently higher percentage of nicotine-cholesterol animals (IV-VI) demonstrated positive electrocardiograms in response to ergonovine than did the cholesterol-alone group (II).

Aortic Pathology. - Results of the gross grading of the aortas are given in Table II. Rabbits in all groups fed cholesterol exhibited marked involvement of the aortas although there was no difference between the cholesterol (II) and any of the nicotine-cholesterol groups (IV-VI). [illegible]

Cardiac Micropathology. - In the control animals (I) there were small amounts of adipose tissue around the coronary vessels. No apparent abnormalities were observed in the walls of any of the blood vessels.

The nicotine group (II) demonstrated minimal paravascular depositions of adipose tissue as well as minimal fatty metamorphosis of myocardial cells. There was, however, in all animals of this group considerable thickening and fibrosis of the small branches of the coronaries.

The cholesterol-only group (III) exhibited somewhat variable changes. In general the coronaries were thickened with advanced atherosclerotic changes. These changes appeared to be most severe in the subendocardial vessels. In some arteries closely adjacent to the endocardium, the lumen was reduced in size to approximately one-tenth the diameter of the vessel. In one animal small areas of necrosis were observed while three animals demonstrated a fatty metamorphosis of the myocardial fibers. [illegible]

The nicotine-plus-cholesterol groups (IV-VI) exhibited atherosclerotic changes essentially similar to those of the cholesterol-alone group (III) with certain notable additions. These additional findings were greater amounts of fatty metamorphosis and the presence of actual early necrosis of the myocardial tissues in all animals. An interaction between the nicotine and cholesterol is suggested as none of the animals fed nicotine alone (II) demonstrated either fatty metamorphosis or myocardial necrosis. Furthermore there was no indication from

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the mortalities or any of the other studies that nicotine alone produced malignant atherosclerosis.

Peripheral Vascular Changes. - Changes in peripheral vascularity appeared in all groups receiving cholesterol. The effect was seen in the limbs as excessive scaling and reddening followed progressively by gross swelling, alopecia localis, suppurative and sometimes bleeding lesions of all four paws and adjacent areas. These changes appeared first in the nicotine-cholesterol groups (IV-VI) between the 12 and 16-week periods and progressed rapidly while in the cholesterol-alone group (III) the lesions did not appear until 22-23 weeks on the diet and then progressed only slowly until the termination of the experiment. The fact that these findings were not seen with the nicotine-alone group (II) once again suggests an interaction between nicotine and cholesterol.

Several animals receiving cholesterol demonstrated a fatty infiltration of the eye. Not only were scleral deposits observed as has been previously reported[1], but the iris was also markedly infiltrated in some animals. There were no apparently significant differences between the cholesterol and nicotine-cholesterol groups in regard to the eye and no attempt had been made to note differences in the times of onset.

Summary:

Groups of female albino rabbits were administered cholesterol, nicotine, and nicotine-cholesterol for a 24-week period. The addition of nicotine to the cholesterol regimen did not significantly affect body weight, serum cholesterol or lipid phosphorus or gross aortic atherosclerosis under the conditions of the test. The nicotine-cholesterol groups demonstrated greater mortality, as well as greater electrocardiographic and pathologic evidence of cardiac involvement and peripheral vascular changes than did the cholesterol or nicotine groups. It is suggested that the combination of nicotine and cholesterol produces a cardiovascular interaction.

[1] RINZLER, S.H., TRAVELL, J., KARP, D., and CHARLESON, D., Detection of coronary atherosclerosis in the living rabbit by the ergonovine stress test, Am. J. Physiol., 184: 605 (1956).

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Table I. - Percentage of Rabbits Exhibiting a Positive Ergonovine Stress Test at 24 Weeks.

Diet	Criterion A[1]	Criterion B[2]
Control	27.3% (3 of 11)	18.2% (2 of 11)
Nicotine	25.0% (3 of 12)	0.0% (0 of 12)
Cholesterol	58.3% (7 of 12)	33.3% (4 of 12)
Cholesterol plus Nicotine "1/8"	71.4% (5 of 7)	71.4% (5 of 7)
Cholesterol plus Nicotine "1/2"	71.4% (5 of 7)	57.2% (4 of 7)
Cholesterol plus Nicotine "2"	72.5% (8 of 11)	54.6% (6 of 11)

[1] Drug-induced depression or elevation of the S-T segment of 1.0 mm. or more below the isoelectric level and/or "flattening" or inversion of the T-wave in lead II, III or IV.

[2] Same as A with the elimination of T-wave "flattening."

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Table II. - Summary of the Gross Grading of the Rabbit Aortas for Atherosclerosis.

Groups	Sample Size	Ascending Aorta	Aortic Arch	Thoracic Aorta	Mean
Control (I)	12	0	.35	.62	.32
Nicotine (II)	12	0	0	0	0
Cholesterol (III)	12	3.59	4.00	2.33	3.30
Cholesterol plus Nicotine "1/8" (IV)	6	3.47	4.00	1.40	2.95
Cholesterol plus Nicotine "1/2" (V)	7	3.66	3.92	2.15	3.24
Cholesterol plus Nicotine "2" (VI)	10	3.91	3.95	2.13	3.33

1. See text for description of the grading scale used.

[illegible]

[illegible] with the elimination of T-wave flattening.

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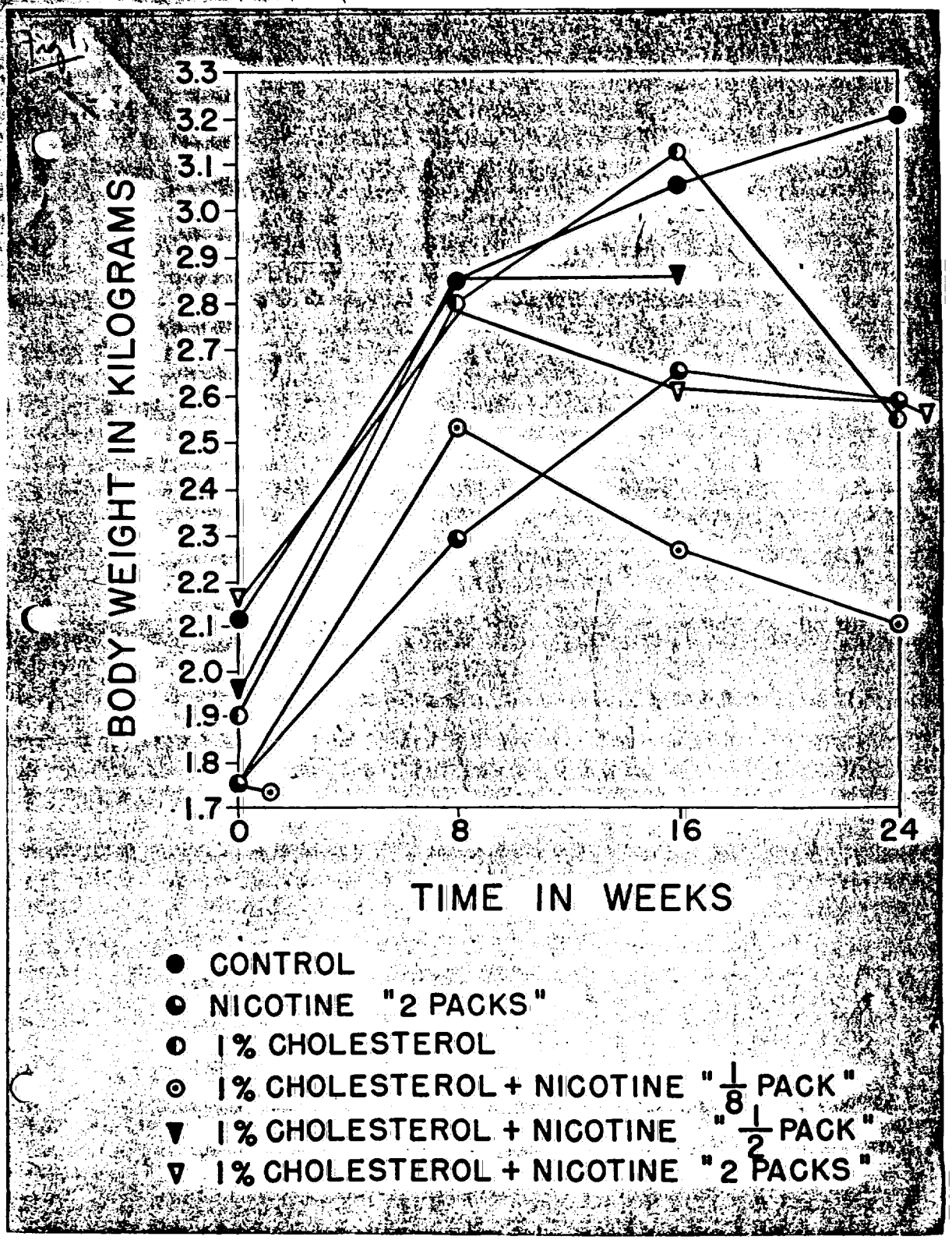

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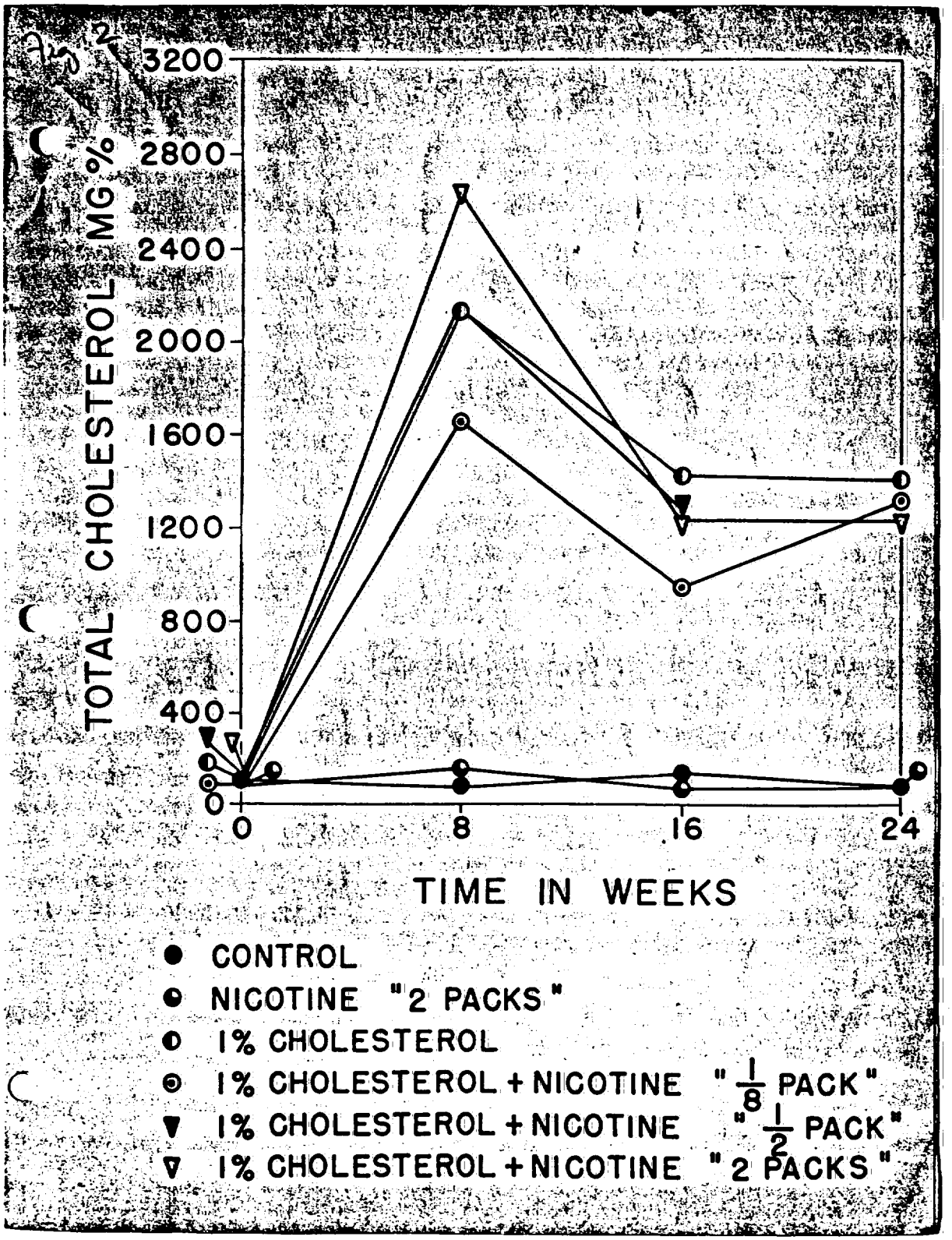

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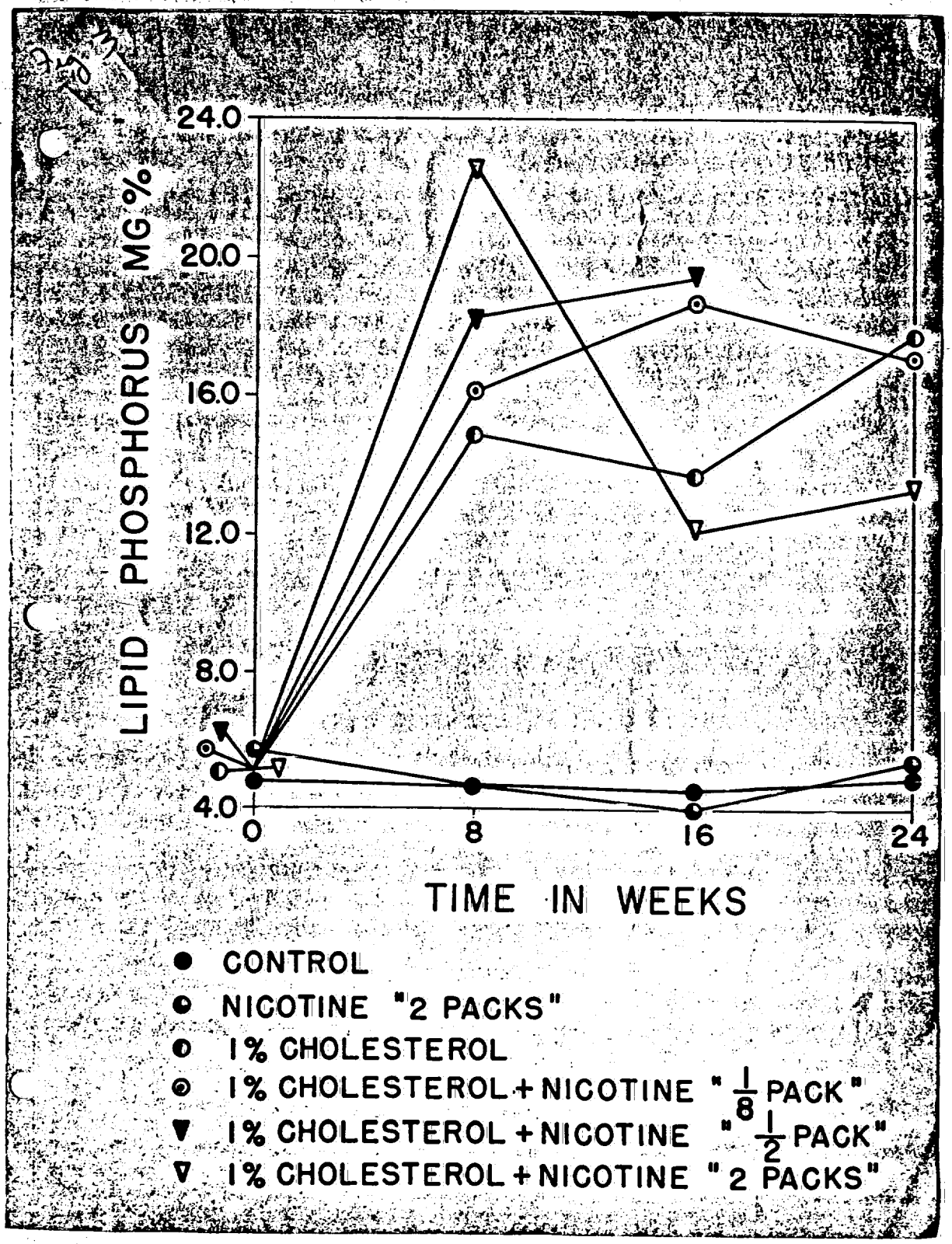

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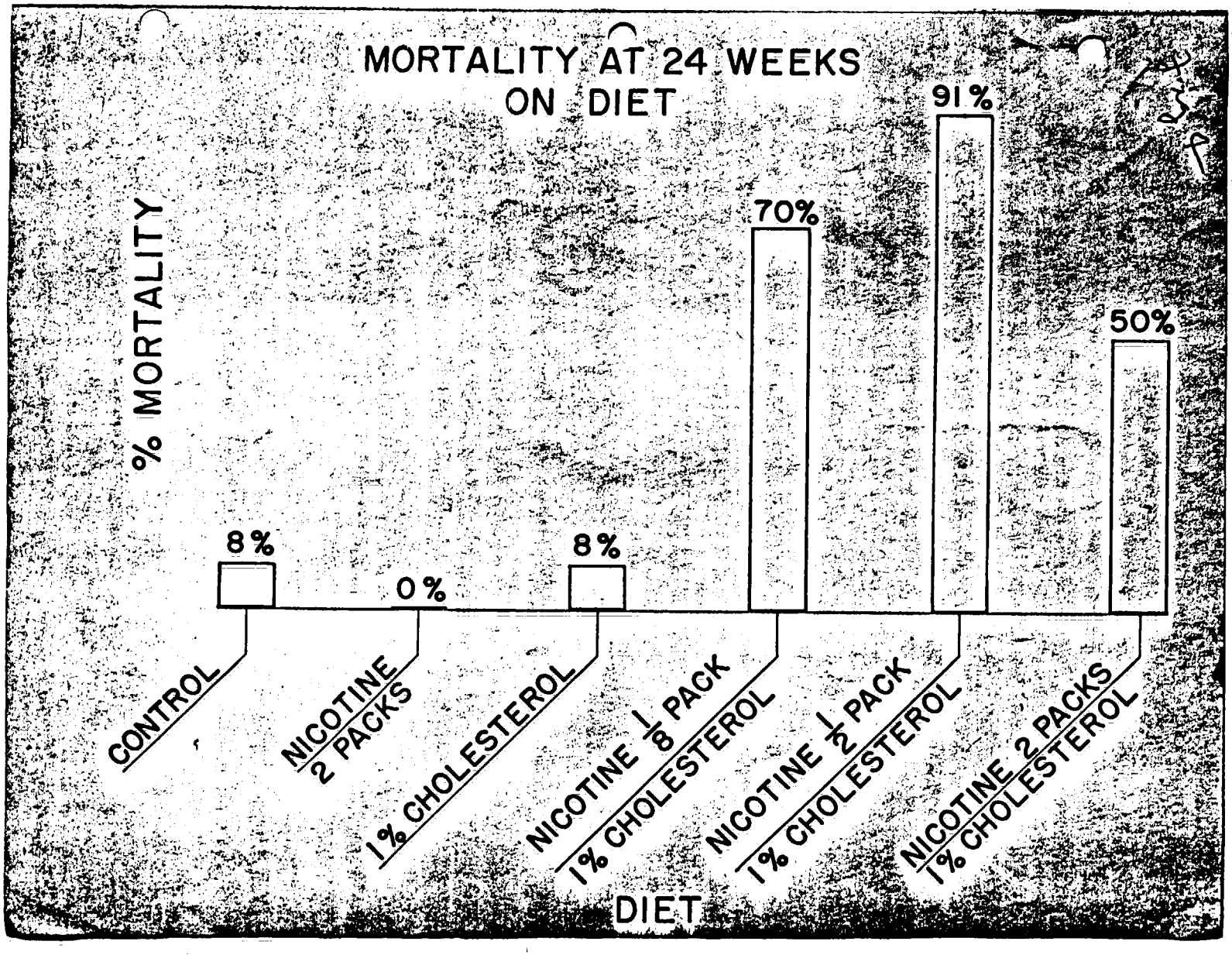

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